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(54) Cosmetic composition containing a plant extracellular matrix extract

(57) The invention provides compositions for the repair and remodelling of sun damaged and aged skin. The composition comprises a component of plant extracellular matrix extract in substantially native conformation, and can include a cosmetic carrier. In particular, the composition can include a glycoprotein of a plant extracellular matrix composition, a carbohydrate polymer of plant extracellular matrix composition, and mixtures thereof, each in substantially native conformation.

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traditionally been divided into primary cell walls that accommodate cell expansion and secondary cell walls that are fully elaborated around expanded cells. Although the relative amounts may vary, the following components are usually present in primary cell wall of higher plants: pectin, xyloglycan, protein, arabinoxylan, β1-3 and β1-4 glucans, cellulose, callose, and lignin (see Roberts, 1989, supra). Grasses (graminaceous monocots) characteristically have low levels of pectin, xyloglycan and protein, and very high levels of arabinoxylan and the glucans (Roberts, 1989, supra).

A number of proteins have been characterized from plant extracellular matrix. Much attention has focused on the hydroxyproline-rich glycoproteins. More recently discovered proteins include repetitive proline-rich proteins, arabinogalactan proteins, extensions, solanaceous lectins, glycine-rich proteins, and thionins (Roberts, 1990, supra). Attachment proteins, analogous to animal integrins, perhaps even containing the fibronectin RGD cell attachment consensus sequence, are also likely present in plant extracellular matrix (see Roberts, 1989, supra).

In cosmetic skin preparations, individual active substances or combinations of isolated individual components of the extracellular matrix of animal origin are often used in the hope of preventing skin aging by substitution of deficient or damaged skin components.

However, cosmetic compositions based on components from animals present serious problems in the form of the potential for transmission of pathogens, in particular viruses and viral-like infectious agents. In that regard, the recent outbreak of bovine spongeform encephalopathy (BSE) in Europe, concentrated primarily in Great Britain, causes great concern, since transmission of the infectious agent through bovine products cannot be excluded. Thus, the art looks to other sources of components for cosmetic compositions.

Hydrolyzed vegetable proteins have attracted attention. Two prominent commercial products are hydrolyzed wheat protein (Vege-Tech®, Glendale CA) and hydrolyzed extensin (hydroxyproline-containing glycoprotein) derived from plants (Provital, S.A.). Thomas (U.S. Patent No. 4,108,849, August 22, 1978) describes alkaline extraction and sterilization, especially autoclaving, of, inter alia, plant tissues or cells for medical and cosmetic purposes.

Therefore, it is an object of the present invention to counteract inadequate cell-matrix interaction, due to aging and ultraviolet light exposure, to repair or remodel the damaged skin which is characterized by damaged dermo-epidermal interface, and to enable normal interactions of epidermis and dermis.

The present invention provides a composition comprising a component of plant extracellular matrix extract in substantially native conformation. The plant extracellular matrix extract can further comprise a glycoprotein of a plant extracellular matrix extract in substantially native conformation, a carbohydrate polymer of a plant extracellular matrix extract in substantially native conformation, and mixtures thereof. The composition can also comprise a cosmetic carrier.

The present invention further provides a method of treating aged or damaged skin, comprising applying a composition comprising a component of plant extracellular matrix extract to the skin. The component of plant extracellular matrix extract for use in the method of treating skin is in substantially native conformation.

The present invention provides a plant-derived cosmetic composition comprising a component of plant extracellular matrix extract in substantially native conformation. Such a component is any molecule that is found in plant extracellular matrix, e.g., a polymer such as a protein, a glycoprotein, a carbohydrate, a proteoglycan, or a mucopolysaccharide. In a preferred embodiment, the composition comprises a glycoprotein of a plant extracellular matrix extract in substantially native conformation. Non-limiting examples of a glycoprotein include a hydroxyproline-rich protein, a repetitive proline-rich protein, an arabinogalactan protein, a lectin, and mixtures thereof. In yet a further embodiment, the composition comprises a carbohydrate polymer of a plant extracellular matrix extract in substantially native conformation. Non-limiting examples of carbohydrate polymers are pectin, xyloglycan, arabinoglycan, glucan, callose, lignin, and mixtures thereof. In yet another embodiment, the composition comprises such a glycoprotein and such a carbohydrate polymer.

As described in section 4.2, infra, the ratio of the several plant extracellular matrix components can vary, depending on the source of the plant extracellular matrix, i.e., what type of plant is used and how the components are obtained.

As used herein, the phrase "plant extracellular matrix extract" indicates that a component normally found in association with plant extracellular matrix lacks the interactions --covalent or otherwise -- normally associating the component with the plant extracellular matrix. In one embodiment, the component of the plant extracellular matrix extract is extracted from plant tissue, which contains the extracellular matrix, thereby releasing the component from the interactions associating it with the extracellular matrix. However, the compositions of the subject invention ar not limited by the method the components are made. For example, rather than extracting the component from plant extracellular matrix, the component can be

although any concentration in the mM range is suitable.

Preferably, the wash solution also contains a preservative, for example, Phenonip at a concentration of about 0.3%. However, any acceptable preservative can be used.

The minced plant tissues are washed extensively to remove impurities and low molecular weight components. In a particular embodiment, the tissues are washed in three changes of wash buffer for about 24 hours each. Preferably the minced plant tissue are constantly and vigorously agitated during the washing steps. Preferably, the washing is done in a cold room, i.e., about 4°C. The ratio of plant tissue to wash solution should be about 1 to 10 (weight to volume), but can range from about 1:3 to about 1:20 (w/v), or within any suitable parameters known to one of ordinary skill. For example, the plant tissue can be washed in more than three changes of wash solution, using less than about a 1:10 ratio of tissue to solution (w/v) in each step. Preferably, as much wash solution as possible is removed after each washing step. In a particular embodiment, the minced tissue is compressed under pressure to remove solution.

After washing with washing solution containing anti-oxidant or anti-peroxide, or both, the plant tissue is washed with the same volume of water, preferably containing a preservative, e.g., Phenonip (0.3%). As much water as possible is removed, e.g., by compression under pressure.

After washing, plant extracellular matrix components for use in the cosmetic compositions are solubilized and extracted from the plant tissue under conditions that preserve the native, biologically functional structure and conformation of the plant extracellular matrix components. Any extraction technique that preserves the functionally active structure of these components may be used; in a particular embodiment, a high concentration salt solution designed to liberate the individual macromolecular components of the tissues may be used. Preferably, the extraction solution contains a preservative, for example, Phenonip at 0.3%.

Salt solutions at moderately low pH are preferred to extract the native molecules into solution, and to avoid degrading the polymers by hydrolysis. In a preferred embodiment, an extraction solution comprising calcium chloride ($CaCl_2$) at a concentration of 0.2 to 1.0 M is used. In another embodiment, guanidine-HCl at a concentration of 0.5 to 2.0 M is used. In yet a further embodiment, both $CaCl_2$ (0.2 to 1.0M) and guanidine-HCl (0.5 to 2.0 M) are used. The relative amounts of plant extracellular matrix components that are extracted depends in part on the type of extraction solution used.

In a particular embodiment, the plant tissue may be treated in a limited way with a proteolytic enzyme, in order to cleave cross-links or cross-linked portions of macromolecules sought for extraction. In particular embodiments pepsin, preferably at a temperature from about 4° to about 18°C (Miller et al., 1972, Biochem 11:4903), or trypsin is used.

The minced, washed plant tissue is extracted with extraction solution preferably but non-limiting in a ratio of about 1:10 (w/v) plant tissue to extraction solution. The ratio may be adjusted depending on the type of plant, the extraction solution used, and whether primary or secondary plant cell wall will be extracted. For example, more solvent may be used with primary tissue (or less with secondary tissue) because more component will be extracted. Preferably, the plant tissues are constantly and vigorously agitated during the extraction period. Extraction continues for about at least 24 hours.

Insoluble material is removed from the extract, for example by compression, and the extract is filtered. In a particular embodiment, a 0.45 µl filter is used.

The plant extracellular matrix composition obtained according to the invention comprises one or more of the following in substantially native conformation: glycoproteins, including hydroxyproline-rich proteins (extensins); repetitive proline-rich proteins, arabinogelactan proteins, and lectins; and carbohydrate polymers such as pectin, xyloglycan, arabinoglycan, glucan, callose and lignin.

The relative proportion of these plant extracellular matrix components in the extract depends upon the source of the extract, i.e., the type of plant used and on the extraction technique employed. For example, an extract of Kudzu leaves contains more hydroxyproline-rich glycoproteins than an extract from maize. However, in each case the components of extracellular matrix have substantially native conformation and are capable of mediating the biological function of the extracellular matrix, and thus are useful for cosmetic compositions.

Extraction and purification of hydroxyproline-rich protein is also described by Hood et al., 1988, Plant Physiol. 87:138-142. Purification of the components of plant extracellular matrix extract is well known in the art.

The component of plant extracellular matrix extract as disclosed herein can be mixed with an acceptable cosmetic carrier to form a cosmetic composition, which can be topically applied to skin. Typical cosmetic carriers for use in the invention include but are not limited to those agents described in Section 4.4, infra.

a)	glycerol monostearate	12.0%
	cetyl stearyl alcohol ethylene oxide adduct containing about 12 mole ethylene oxide	1.5%
	cetyl stearyl alcohol ethylene oxide adduct containing about 20 mole ethylene oxide	1.5%
	cetyl alcohol	2.0%
	2-octyl-dodecanol	10.0%
	isoctyl stearate	8.0%
	caprylic/capric acid triglyceride	3.0%
	methylparaben	0.17%
	propylparaben	0.03%
	and	
b)	water, distilled	46.8%
	glycerol	5.0%
	and	
c)	plant extracellular matrix extract according to the present invention (prepared as explained above)	10.0%

Mixture a) is heated to approximately 70 °C and mixture b) is likewise heated to approximately 70 °C and then added while stirring to mixture a).

Stirring is continued until the cream has cooled down to approximately 30 °C. Then composition c) is added while stirring and the cream is homogenized.

By the term cream used herein are meant all cosmetic materials which include, for instance, hand creams, cleansing creams, milky lotions, cold creams, vanishing creams, hair creams, foundation creams, beauty washes, facial packs and the like.

Example 2: EMULSION

Oil-in-water emulsion (o/w) containing the active composition (the plant extracellular matrix extract prepared according to the present invention) comprising:

a)	glycerol monostearate	3.0%
	cetyl stearyl alcohol	2.0%
	cetyl stearyl alcohol ethylene oxide adduct containing about 12 mole ethylene oxide	1.5%
	cetyl stearyl alcohol ethylene oxide/adduct containing about 20 mole ethylene oxide	1.5%
	glycerol monooleate	0.5%
	2-octyl-dodecanol	10.0%
	methylparaben	0.17%
	propylparaben	0.03%
	and	
b)	water, distilled	66.3%
	glycerol	5.0%
	and	
c)	plant extracellular matrix extract according to the present invention	10.0%

Mixture a) is heated to approximately 70 °C and mixture b) is likewise heated to approximately 70 °C and added while stirring to mixture a).

Stirring is continued until the o/w emulsion has cooled down to approximately 30 °C. Then composition c) is added while stirring and the o/w emulsion is homogenized.

Example 3: GEL

A gel containing the active composition (plant extracellular matrix extract prepared according to the present invention) comprising:

Sodium metabisulfite was used as an antioxidant to reduce oxidation of phenols and plant pigments. Sodium metabisulfite also inhibits the release of hydrogen peroxide, which occurs when plant tissues are injured. After the three washing steps, the material was washed with the same amount of water containing 0.3 % Phenonip to remove the sodium metabisulfite.

Subsequently, the residue containing the minced soybean was extracted with 1.5 l of 0.2 M $CaCl_2$-solution for at least 24 hrs. Finally, the insoluble material was removed by filter compression and the extract filtered through a 0.45 µ (Millipore HVLP 14250) filter.

The matrix extract collected from soybean contained about 1 % native plant proteins, as determined by the Lowry method (Sigma Chemicals Co.). The hydroxyproline concentration was 18 µg/ml.

EXAMPLE 7:RAT SKIN TEST

A double blind study was carried out using rats whose skins were irradiated with UV-radiation for 1 month according to the following protocol:

C: Normal untreated animals (no UV radiation)

K: Control animal treated with a cream composition containing no active ingredient and subjected to 1 month UV radiation.

S: Animals treated with a cream composition containing a soybean matrix composition of the present invention and subjected to 1 month UV radiation.

The parameters measured included the measurement of the soluble collagen content which decreases with age as well as the effect on the elasticity of the skin in terms of the increase in stretch resistance, which increases with age.

	C	K	S
% soluble collagen	67.01	58.94	64.01
stretch resistance*	1.977	2.367	2.001

*) These values have no dimensions as they are determined as a coefficient of an equation.

The research carried out above was a double blind study, which indicates that skin damages which are analogous to aging may be best restituted by the active ingredient S contained in the composition, i.e. the composition of the invention. It can further be concluded that damages from UV light caused by treatment over a prolonged period of time can be completely eliminated, opening up particularly interesting possibilities of application in view of the present increased risks due to UV radiation from sunlight (unproprotionally high UVB portion).

EXAMPLE 8: A CLINICAL TEST

Marked areas of the dorsal (back of hand) skin of volunteers having clinical signs of aging skin are treated with the following test creams for four weeks, twice a day:

a) cream (oil-in-water) having 10% of the active composition (plant extracellular matrix extract prepared according to the present invention);

b) cream base (oil-in-water) as control.

The skin is evaluated for improvement in luminosity, moisturization, satinity and elasticity, and reduction of visible signs of aging and of the depth of wrinkles and fine lines.

This experiment can show and establish that the topical application of the agent of the present invention results in improved luminosity, moisturization, satinity and elasticity of the skin. Visible signs of aging are reduced and the depth of the wrinkles and fine lines is reduced.

Claims

1. A composition comprising a plant extracellular matrix extract, comprising a glycoprotein in substantially native conformation; a carbohydrate polymer in substantially native confirmation and an arabinogalactan protein in substantially native conformation and a cosmetic carrier.

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EUROPEAN SEARCH REPORT

Application Number
EP 94 10 2578

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	EP-A-0 532 465 (PENTAPHARM AG) 17 March 1993 * page 3, line 6 - line 44 * * page 4; example 1 * * page 5; example 3 * * claim 3 *	1-16	A61K7/48
A	EP-A-0 314 835 (FAHIM MOSTAFA) 10 May 1989 * page 2, line 20 - line 35 * * page 2, line 51 - line 53 * * page 9; example 4 *	1-16	
A	WO-A-86 01713 (INNOFINANCE ALTALANOS INNOVACIOS PENZINTEZET) 27 March 1986 * page 6; example 7 * * claims 1,7 *	1-16	
A	WO-A-89 05137 (KLUDAS M.) 15 June 1989 * claims 1,2 *	1-16	
D,A	FR-A-2 261 755 (THOMAS A.) 19 September 1975 & US-A-4108849 * claims 1-11 *	1-16	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
A	CHEMICAL ABSTRACTS, vol. 105, no. 15, 13 October 1986, Columbus, Ohio, US; abstract no. 130734, ASAMIZU T. ET AL 'Glycoprotein associated with the cell wall of carrot cells in suspension culture' * abstract * & SHOKUBUTSU SOSHIKI BAIYO vol. 3, no. 1 , 1986	1-16	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	25 July 1994	Boulois, D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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